



# The Big Picture

*Specific Aims*

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# Session Plan

- Discuss the importance and purpose of the Specific Aims page.
- Describe a framework for organizing the Specific Aims page based on its components.
- Dissect a sample Specific Aims page.



**How Important is  
the Specific Aims Page?**



# The Specific Aims page...

- ▶ **Is the most important part of your application!**
- ▶ Outlines the “big picture” of your project.
- ▶ Is your primary marketing document.



## Therefore, the Specific Aims page must...

- ▶ Be compelling.
- ▶ Excite!
- ▶ Move the reviewers to be your advocate.

Create enthusiasm!



**It also must touch on all review criteria.**

- Significance
- Innovation
- Investigator
- Approach
- Environment

## Overall Impact

The likelihood for the project to exert a **sustained, powerful influence** on the research field(s) involved, in consideration of the five core review criteria.



**Preparing to Write  
the Specific Aims Page**



# Where to Start

- Always consult the guidelines (funding announcement).
  - [SF424 \(R&R\)- Forms Version I](#)
- You must have well-defined aims!
- The **content** of the Specific Aims page **derives** largely from the **Significance** and **Innovation** sections and from **Preliminary Studies**.
  - **Write the Significance and Innovation sections first.**
- Recognize that refining the aims is an iterative process.



# NIH Guideline for Specific Aims Page

- ▶ State concisely the **goals** of the proposed research and summarize the **expected outcome(s)**, including the **impact** that the results of the proposed research will exert on the **research field(s) involved**.
- ▶ List succinctly the **specific objectives** of the research proposed (e.g., to test a stated hypothesis, create a novel design, solve a specific problem, challenge an existing paradigm or clinical practice, **address a critical barrier to progress in the field**, or develop new technology).



# Use the Aims Worksheet

We have provided an Aims worksheet to help you build the Specific Aims page.

- The worksheet is comprised of questions derived from the NIH review criteria.
- In completing the worksheet, you will identify, articulate, and organize the issues that are critical for the review of your proposal.



# Writing the Specific Aims Page



# Framework for the Specific Aims Page



**Overarching Problem (Big Picture) & Overall Goal**

**Context & Setting**

**Central Hypothesis**

**Specific Aims &  
Experimental Overview**

**Expected  
Outcomes**

**Impact**

(Follows the Aims Worksheet!)



Let's review each piece of the framework in more detail...



Specific Aims Page Framework

# Overarching Problem and Overall Goal



**Overarching Problem (Big Picture) & Overall Goal**

Context & Setting

Central Hypothesis

Specific Aims &  
Experimental Overview

Expected  
Outcomes

Impact



Specific Aims Page Framework

# Overarching Problem and Overall Goal

- ▶ In a few interest-grabbing sentences, define the big picture
  - ▶ Overarching public health problem/focus of your work.
  - ▶ Your goal (long-term or immediate) with respect to the problem.
  - ▶ Specific aspect of the problem addressed by your current proposal.

Specific Aims Page Framework

# Context and Setting

Overarching Problem (Big Picture) & Overall Goal

**Context & Setting**

Central Hypothesis

Specific Aims &  
Experimental Overview

Expected  
Outcomes

Impact

## **Context and Setting**

- Briefly, summarize the current state of knowledge regarding the goal of your current proposal, drawing on/evaluating the contributions of others and your own work/preliminary studies.
- Define the specific gap in knowledge/challenge impeding further progress that your proposal will address.
- Define the importance of addressing this specific gap/challenge to human health and to advancing your field of research.



Specific Aims Page Framework

# Central Hypothesis



Overarching Problem (Big Picture) & Overall Goal

Context & Setting


**Central Hypothesis**

Specific Aims &  
Experimental Overview

Expected  
Outcomes

Impact

## **Central Hypothesis**

- ▶ State the overall hypothesis, derived from preliminary data, that the studies proposed in your Specific Aims will test, with the objective of filling the gap.
  - ▶ The overall hypothesis should encompass all aims and should relate directly to the overall goal and public health problem.
- 



Specific Aims Page Framework

# Specific Aims & Experimental Overview



Overarching Problem (Big Picture) & Overall Goal

Context & Setting

Central Hypothesis

**Specific Aims &  
Experimental Overview**

Expected  
Outcomes

Impact



Specific Aims Page Framework

## **Specific Aims & Experimental Overview**

- Define at least 2 and no more than 4 specific aims, beginning each with a verb (e.g., determine, evaluate, identify, explore).
- For each aim, very briefly describe the general experimental design and/or methods you will use.



## Specific Aims Page Framework

### ***Specific Aims...***

- ▶ Are concrete, well-focused **objectives** that flow logically from your hypothesis and are intended to test its validity.
- ▶ Are interconnected, but not interdependent.
- ▶ Have clear endpoints—achievable within the time frame of your proposal—that reviewers can easily assess.
- ▶ Should demonstrate advancement in your work.



## Specific Aims Page Framework

# ***Wording the Aims***

### Too vague

- Specific Aim: Conduct statistical analysis on already collected data.

### Too detailed

- Specific Aim 1. Correlate urinary concentration of X with food consumption patterns available through a questionnaire, thereby identifying foods that are major contributors of X.

### Just right

- Aim 2. Investigate the effects of SON E upregulation on gene expression, cell proliferation, and self-renewal in hematopoietic stem cells.



Specific Aims Page Framework

# Expected Outcomes and Impact



Overarching Problem (Big Picture) & Overall Goal

Context & Setting

Central Hypothesis

Specific Aims &  
Experimental Overview

**Expected  
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**Impact**

## Expected Outcomes and Impact

- Define the expected impact of your success in achieving the goals of your proposal.
- In other words, what will be possible/known that was not possible/known before, with respect to
  - Human health and disease?
  - Advancement of your field of research?

Relate this to the **overarching problem** stated at the beginning of the Aims page.




# **Sample Specific Aims Page**



# Specific Aims Page: Example

- ▶ Peter John Myler, PhD, and Marilyn Parsons, PhD  
“Ribosome profiling of *Trypanosoma brucei*” (R21)



Overarching problem  
(big picture),  
Context and setting  
(preliminary data)

Gap in knowledge,  
Hypothesis,  
Overview of approach

Specific Aims,  
Experimental overview

Expected outcomes  
and impact

## 2. Specific Aims

Gene expression in trypanosomatids (such as *Trypanosoma brucei* and the various *Leishmania* species) is distinct from other well-studied eukaryotes because the protein-coding genes are transcribed polycistronically. However, co-transcribed mRNAs encode proteins that display dramatic variation in abundance both within and across developmental stages, indicating that post-transcriptional controls provide the major means of regulating expression of individual genes. Our previous microarray study has shown significant differences in mRNA abundance within and across *T. brucei* bloodstream and insect stages (likely reflecting differences in mRNA stability), while other studies have identified considerable changes in the proteome. A recent global analysis of mRNA levels and protein abundances (from the same biological samples) at several time-points during promastigote-to-amastigote differentiation of *L. donovani* (conducted by the Myler lab) showed that the correlation between these is rather low. However, both microarrays and proteomic analysis are limited by a lack of resolution in quantitation of lower abundance molecules, leaving the true correlation between mRNA and protein levels open to question. Furthermore, other data suggests that translational and/or post-translational controls also play significant roles. For example, in-depth analysis (by the Parsons lab) of two *T. brucei* genes demonstrated translational control as a key mechanism. We therefore hypothesize that translational controls function both to tune the levels of protein within stages and to change the levels across stages. This project seeks to address this hypothesis by quantitatively assessing the rate at which cellular mRNAs are being actively translated at any particular time. This will be accomplished by adapting and applying a recently-described technology that couples the ability to isolate the specific “footprints” of mRNAs that are occupied by ribosomes (an indicator of translation) with the depth and breadth of next generation sequencing (NGS). To establish the system and test our hypothesis for *T. brucei*, we propose the following Specific Aims.

**Aim 1. Establish the ribosome protection assay in *T. brucei* strain 927 cultured procyclic forms.** Optimization of footprinting, library construction and informatics will be done using cultured log-phase procyclic forms, which are readily available under standardized conditions. Cell lysates will be treated with RNase I and ribosome-protected RNA fragments will be isolated and used to generate libraries for sequencing *via* Illumina NGS technology. The resulting data will be entered into our RNA-seq pipeline and aligned with the *T. brucei* genome to identify the number and location of ribosomes that are bound to gene-specific mRNA. This data will indicate the level of gene-specific translation for every gene detected, as well as identifying the specific sequences on each mRNA that are translated. Comparison with the profile of total cellular mRNA will establish the translational efficiency of transcripts corresponding to specific genes.

**Aim 2. Identify genes that are regulated at the level of translation during *T. brucei* development.** We will carry out similar studies on rapidly-dividing, mammalian-infective slender bloodstream forms and non-dividing stumpy bloodstream forms from animals. Comparison of the ribosome profile of mRNAs at these stages and that of procyclic forms (from Aim 1) will identify genes that are regulated at the level of translation.

The proposed work promises to provide an important new tool for studying trypanosomatid gene expression, yielding clues to the mechanism of translational control in trypanosomatids, and new information on the extent of translation of individual gene products. In addition, it should resolve the current debate over the function of the numerous recently identified RNAs that contain only short open-reading frames, and has the potential to identify non-canonical open-reading frames, thus significantly enhancing the ongoing genome annotation. We also anticipate that this technology will be very useful to those researchers wishing to determine which trypanosomatid proteins are likely to be present in infective stages, and thus might serve as drug and vaccine targets.



Specific Aims Page: Example

## Overarching Problem (“Big Picture”)

Gene expression in trypanosomatids (such as *Trypanosoma brucei* and the various *Leishmania* species) is distinct from other well-studied eukaryotes because the protein-coding genes are transcribed polycistronically. However, co-transcribed mRNAs encode proteins that display dramatic variation in abundance both within and across developmental stages, indicating that post-transcriptional controls provide the major means of regulating expression of individual genes.

→ Proteins vary in abundance within and across developmental stages despite genes being transcribed polycistronically, indicating that gene expression is regulated post-transcriptionally



Specific Aims Page: Example

## Context and Setting (Preliminary Data)

Our previous microarray study showed significant differences in mRNA abundance within and across *T. brucei* bloodstream and insect stages (likely reflecting differences in mRNA stability), while other studies have identified considerable changes in the proteome. A recent global analysis of mRNA levels and protein abundances (from the same biological samples) at several time-points during promastigote-to-amastigote differentiation of *L. donovani* (conducted by the Myler lab) showed that the correlation between these is rather low.

→There is a discrepancy between mRNA and protein levels



Specific Aims Page: Example

## Specific Gap in Knowledge

However, both microarrays and proteomic analysis are limited by a lack of resolution in quantitation of lower abundance molecules, leaving the true correlation between mRNA and protein levels open to question. Furthermore, other data suggests that translational and/or post-translational controls also play significant roles. For example, in-depth analysis (by the Parsons lab) of two *T. brucei* genes demonstrated translational control as a key mechanism.

→ Gap in knowledge on role of translational/post-translational regulation



Specific Aims Page: Example

## **Hypothesis/Project's Goal**


We therefore hypothesize that translational controls function both to tune the levels of protein within stages and to change the levels across stages. This project seeks to address this hypothesis by quantitatively assessing the rate at which cellular mRNAs are being actively translated at any particular time.



Specific Aims Page: Example

## **Overview of Approach**

This will be accomplished by adapting and applying a recently described technology that couples the ability to isolate the specific “footprints” of mRNAs that are occupied by ribosomes (an indicator of translation) with the depth and breadth of next-generation sequencing (NGS).





Specific Aims Page: Example

## **Specific Aims/Experimental Design**

To establish the system and test our hypothesis for *T. brucei*, we propose the following Specific Aims.

### **Aim 1. Establish the ribosome protection assay in *T. brucei* strain 927 cultured procyclic forms.**

Optimization of footprinting, library construction and informatics will be done using cultured log-phase procyclic forms, which are readily available under standardized conditions. Cell lysates will be treated with RNase I, and ribosome-protected RNA fragments will be isolated and used to generate libraries for sequencing via Illumina NGS technology. The resulting data will be entered into our RNA-seq pipeline and aligned with the *T. brucei* genome to identify the number and location of ribosomes that are bound to gene-specific mRNA. This data will indicate the level of gene-specific translation for every gene detected, as well as identifying the specific sequences on each mRNA that are translated. Comparison with the profile of total cellular mRNA will establish the translational efficiency of transcripts corresponding to specific genes.



Specific Aims Page: Example

## **Specific Aims/Experimental Design**

**Aim 2. Identify genes that are regulated at the level of translation during *T. brucei* development.**

We will carry out similar studies on rapidly dividing, mammalian-infective slender bloodstream forms and non-dividing stumpy bloodstream forms from animals. Comparison of the ribosome profile of mRNAs at these stages and that of procyclic forms (from Aim 1) will identify genes that are regulated at the level of translation.



Specific Aims Page: Example

## **Expected Outcomes and Impact**

The proposed work promises to provide an important new tool for studying trypanosomatid gene expression, yielding clues to the mechanism of translational control in trypanosomatids, and new information on the extent of translation of individual gene products. In addition, it should resolve the current debate over the function of the numerous recently identified RNAs that contain only short open-reading frames, and it has the potential to identify non-canonical open-reading frames, thus significantly enhancing the ongoing genome annotation. We also anticipate that this technology will be useful to researchers who wish to determine which trypanosomatid proteins are likely to be present in infective stages and, thus, might serve as drug and vaccine targets.



# Recap

- The Specific Aims page must...
  - Present an overview of the whole project.
  - Create enthusiasm for the project.
  - Touch on all review criteria, including Overall Impact.
- Use the worksheet!
- Continually revise Specific Aims as you develop the proposal.